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(19) (CA) **CANADIAN PATENT** (12)

(54) Polymers Containing Thiol Groups, a Process for their Preparation, and their Use

(72) Stüber, Werner , Germany (Federal Republic of)
Löbermann, Hartmut , Germany (Federal Republic of)

(73) Behringwerke Aktiengesellschaft , Germany (Federal Republic of)

(30) (DE) Germany (Federal Republic of) P 37 26 454.0
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Abstract of the Disclosure


Polymers containing thiol groups are described which can be obtained by introducing oxirane groups into polymers containing hydroxyl groups and treating the product with a salt of hydrogen sulfide.

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Polymers containing thiol groups,
a process for their preparation, and their use

The invention relates to polymers containing thiol groups,
a process for their preparation, and their use.

- 5 Polymeric carrier materials which are derivatized by mercapto groups are of great interest since, on the one hand, substances which usually interact with sulfhydryl groups are purified using them and, on the other hand, substances can be irreversibly immobilized on sulfhydryl
- 10 groups. Such substances can be, for example, enzymes, antibodies, proenzymes or antigens. Hitherto, derivatives of agarose, in particular, have been employed as the carrier matrix. During the preparation of these polymeric carriers, the chemical introduction of the
- 15 sulfhydryl functions deserves particular attention. Polymer derivatizations using homocystein thiolactone, glutathione coupling reactions with prior cyanogen bromide activation, and activation using epichlorohydrin and subsequent reaction with thiosulfate along with a
- 20 reduction step, and reaction of an oxirane group with dithiothreitol are known and thus correspond to the prior art (R. Axén et al., Acta Chemica Scan. B 29, 471-474, 1975).

- 25 However, these thiol-containing polymers and their preparation processes are afflicted with disadvantages and are thus capable of improvement. The reasons for this are, on the one hand, in the matrix, which, due to the carbohydrate structure, is mechanically sensitive and can also be attacked by microbes, and, on the other hand, are
- 30 in the process for introduction of the sulfhydryl group, which is either expensive or inconvenient, and in some cases also includes the production of unstable bonds.
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The object of the invention was thus to develop a process which allows polymers containing thiol groups to be prepared simply and economically. In addition, the condition must be fulfilled that the chemical bonds produced
5 are distinguished by high stability.

The object of the invention was also to prepare thiol-containing polymers which, as a consequence of the matrix employed for derivatization, are distinguished by superior mechanical and chemical stability.

10 Surprisingly, it has now been found that polymers which were derivatized using oxirane groups reacted with water-soluble salts of hydrogen sulfide to form immobilized sulfhydryl functions.

The invention relates to a polymer containing thiol
15 groups which is obtainable by introducing oxirane groups into a water-insoluble polymer containing hydroxyl groups and treating the polymer containing oxirane groups with a water-soluble salt of hydrogen sulfide, preferably an alkali metal salt or alkaline earth metal salt.

20 Suitable polymers containing hydroxyl groups are carbohydrate-based polymers, for example agarose gels or dextran gels, or polymers based on methacrylamide, N-methylene-bis-methacrylamide, glycidyl methacrylate/polyethylene glycol derivatives and pentaerythritol
25 dimethacrylate.

Polymers which, as a consequence of their chemical nature, are known as copolymers and constructed from acrylic acid derivatives or methacrylic acid derivatives and are provided with oxirane groups are suitable in a
30 preferred manner for the preparation of polymers containing thiol groups. The monomer components of such copolymers are, for example, methacrylamide, N-methylene-bis-methacrylamide, glycidyl methacrylates, glycidyl

methacrylate/polyethylene glycol derivatives or pentaerythritol methacrylic acid derivatives. In particular, resins which are commercially available under the names ^RFractogel or ^RSepabeads are suitable.

- 5 The hydroxyl groups of these water-insoluble resins are reacted by known methods, for example in accordance with EP-A-0,203,463, with 1-chloro-2,3-epoxypropane (epichlorohydrin) or with 1,4-bis(2,3-epoxypropoxy)butane, and oxirane groups are thus introduced into the polymers.
- 10 The epoxidization is carried out, for example, by suspending 1 kg of water-moist polymer in 1,500 ml of water and 650 ml of 2 N sodium hydroxide solution and reacting the suspension with 200 ml of epichlorohydrin at elevated temperature, for example 40-50°C. After 1-4 hours,
- 15 the polymer is washed with water and sucked dry.

- The oxirane-containing polymer is then taken up in water or an aqueous buffer solution, 500 ml to 3 l, preferably about 1 l, usually being added per kg of resin. Buffers which can be employed are salt solutions, for example
- 20 sodium phosphate buffer, sodium citrate buffer or HEPES buffer, having a pH of 4 to pH 13. 1 liter of a 0.25 molar sodium hydrogen carbonate solution is preferably added per kg of polymer.

- 10-200 g of a water-soluble sulfide or hydrogen sulfide, preferably an alkali metal sulfide, are then added.
- 25 Particularly preferably, 70-80 g of sodium sulfide x 9H₂O are added, and the batch is stirred at a temperature of 0-70°C, preferably 35-45°C. The reaction time chosen is 2 to 48 hours, preferably 12-14 hours. The
- 30 polymer is then washed with water and/or 0.5 ml/100 ml strength acetic acid.

The invention also relates to a process for the preparation of a polymer containing thiol groups, wherein a

water-insoluble polymer containing hydroxyl groups is reacted for one to four hours at 40-50°C with 1-chloro-2,3-epoxypropane (epichlorohydrin) or 1,4-bis(2,3-epoxypropoxy)butane, whereby oxirane groups are introduced into the polymer, the polymer is washed with water, taken up in aqueous solution and treated for 2 to 48 hours at a temperature of 0-70°C with a water-soluble sulfide, and the polymer is washed with water or dilute acid.

10 A preferred polymer according to the invention is one which can be obtained by introducing oxirane groups, by reaction with epichlorohydrin, into a copolymer prepared from pentaerythritol, methacrylic acid derivatives and polyethylene glycol and which is crosslinked with divinylbenzene and treating with sodium sulfide.

15 A further preferred polymer according to the invention is one which can be obtained by introducing oxirane groups into a polymer based on methacrylic acid derivatives (^RSepabeads) by reaction with butanediol diglycidyl ether, and treating with sodium sulfide.

20 The polymeric carriers which are provided with thiol functions by the above process are very particularly preferably suitable, due to their mechanical and chemical stability, for immobilization of enzymes, proenzymes, antibodies or antigens and for chromatography of substances which interact with mercapto groups.

25 The thiol-containing polymers prepared in this way are furthermore suitable, after prior activation using a disulfide, for reaction with thiol-containing substances, for example proteins, with disulfide exchange. Such proteins can thereby be concentrated or eluted from the polymer and thus purified. Particularly suitable disulfides for activation of polymeric mercapto groups are, for example, 2,2'-dipyridyl disulfide; bis-(2-pyridyl-N-oxide) disulfide and 5,5'-dithiobis-(2-nitrobenzoic acid).

A polymer according to the invention can preferably be used for purification of α_1 -antitrypsin.

The examples below illustrate the invention.

Example 1

- 5 Preparation of a thiol-containing polymer based on
R_{Fractogel}

1,000 ml of R_{Fractogel} HW-65 (F), a copolymer prepared from pentaerythritol, methacrylic acid derivatives and polyethylene glycol (manufacturer: Toyo Soda), were
10 treated with 350 ml of water and 275 ml of 5 N sodium hydroxide solution, and the mixture was warmed to 45°C. 200 ml of epichlorohydrin were added, and the mixture was stirred at this temperature for 2 hours. The mixture was filtered and washed with water until the pH had dropped
15 to 7. The suction-dried resin was taken up in 1,400 ml of 0.2 molar sodium hydrogen carbonate solution and treated with 72 g of Na₂S x 9H₂O. The batch was stirred at 40°C for 15 hours, filtered under suction and washed thoroughly with water and subsequently with 0.5 ml/100 ml
20 strength acetic acid.

Example 2

- Preparation of a thiol-containing polymer based on
R_{Sepabeads} (manufacturer: Mitsubishi)

1,000 ml of R_{Sepabeads} HG05 were suspended in 1,000 ml
25 of 0.6 N NaOH containing 2 g of NaBH₄. 1,000 ml of butanediol diglycidyl ether were subsequently added, and the suspension was stirred at room temperature for 8 hours. The epoxy-activated R_{Sepabeads} HG05 material was subsequently washed with 15 l of water on a glass frit.
30 The reaction with Na₂S x 9H₂O takes place as described in Example 1.

Example 3

Activation of thiol-containing ^RFractogel using 2,2'-dithiobis(pyridine N-oxide)

500 ml of thiol-containing ^RFractogel HW-65, which was prepared as described in Example 1, was equilibrated with a buffer A containing 50 mmol/L of tris/HCl and 150 mmol/L of NaCl, pH 7.5. The resin material was subsequently incubated 4 times for 320 minutes at room temperature with 1 L of buffer A + 1.5 mmol/L of 2,2'-dithiobis-(pyridine N-oxide) in each case and then again washed with buffer A. The resin material activated in this way can now be used, for example, for disulfide exchange reactions.

Example 4

15 Activation of thiol-containing ^RSepabeads HG05 using di-(2-pyridyl) disulfide

500 ml of thiol-containing ^RSepabeads HG05, which were prepared as described in Example 2, were activated using 1.5 mmol/L of di-(2-pyridyl) disulfide under the conditions of Example 3.

Example 5

Purification of alpha₁-antitrypsin

500 ml of human plasma were diluted with 1,000 ml of water containing 30 mmol/L of mercaptoethanol, and the mixture was incubated at room temperature for 1 hour and subsequently subjected to fractional ammonium sulfate precipitation between 50% saturation (w/v) and 75% saturation (w/v). The 50%-75% (w/v) residue was dissolved in a solution containing 50 mmol/L of Tris, 200 mmol/L of NaCl and 1 mmol/L of EDTA, pH 8.0, and

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chromatographed over a 500 ml ^RACA 202 column. The fractions containing alpha₁-antitrypsin were purified and transferred to a thiol-^RFractogel column (100 ml) activated using 2,2'-dithiobis(pyridine N-oxide). The
5 column was washed with the same buffer, and alpha₁-antitrypsin was eluted by means of a solution containing 10 mmol/L of dithiothreitol. The alpha₁-antitrypsin thus obtained was substantially freed from impurities.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A polymer containing thiol groups which is obtainable by introducing oxirane groups into a water-insoluble polymer containing hydroxyl groups and treating the polymer containing oxirane groups with a water-soluble salt of hydrogen sulfide.
2. A polymer as claimed in claim 1, wherein the polymer containing hydroxyl groups is a carbohydrate-based polymer or a polymer based on methacrylamide, N-methylene-bis-methacrylamide, glycidyl methacrylate/polyethylene glycol derivatives or pentaerythritol dimethacrylate.
3. A polymer as claimed in claim 1, wherein the polymer containing hydroxyl groups is an agarose gel.
4. A polymer as claimed in claim 1, wherein the salt of hydrogen sulfide is an alkali metal salt or an alkaline earth metal salt.
5. A polymer as claimed in claim 1, wherein the polymer containing oxirane groups contains acrylic acid derivatives or methacrylic acid derivatives.
6. A polymer as claimed in claim 1, wherein the water-insoluble polymer containing hydroxyl groups is a copolymer prepared from pentaerythritol, methacrylic acid derivatives and polyethylene glycol and which is crosslinked with divinylbenzene, wherein the oxirane groups are introduced by reacting this copolymer with epichlorohydrin, and wherein it is treated with sodium sulfide.
7. A polymer as claimed in claim 1, wherein the water-insoluble polymer containing hydroxyl groups is a polymer based on methacrylic acid derivatives

(^RSepabeads), wherein the oxirane groups are introduced by reacting this polymer with butanediol diglycidyl ether, and wherein it is treated with sodium sulfide.

8. A process for the preparation of a polymer containing thiol groups, wherein a water-insoluble polymer containing hydroxyl groups is reacted for one to four hours at 40-50°C with 1-chloro-2,3-epoxypropane (epichlorohydrin) or 1,4-bis(2,3-epoxypropoxy)butane, whereby oxirane groups are introduced into the polymer, the polymer is washed with water, taken up in aqueous solution and treated for 2 to 48 hours at a temperature of 0-70°C with a water-soluble sulfide, and the polymer is washed with water or dilute acid.
 9. The use of a polymer as claimed in claim 1 for immobilization of enzymes, proenzymes, antibodies or antigens and for chromatography of substances which interact with mercapto groups.
 10. The use of a polymer as claimed in claim 1, after activation using a disulfide, for binding substances containing thiol groups.
 11. The use of a polymer as claimed in claim 1 for purification of alpha₁-antitrypsin.
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REMPLACEMENT

SECTION is not Present
Cette Section est Absente